



07648.0023-00000

CLAIMS

1. A relational database comprising the data of Table I.
2. A method of staging embryos comprising:
 - a) providing at least one embryo;
 - b) detecting the expression in the embryo of at least one RNA transcript of Table I; and
 - c) correlating the expression of said transcript to one or more embryonic stages.
3. The method of claim 2 wherein at least two RNA transcripts are detected or determined and correlated to one or more embryonic stages.
4. The method of claim 2 wherein expression of the at least one RNA transcript is analyzed by hybridization with at least one probe of Table I.
5. The method of claim 2 wherein expression of the at least one RNA transcript is analyzed by hybridization with a variant of at least one probe of Table I.
6. The method of claim 5 wherein said variant hybridizes to at least one probe of Table I under conditions of high stringency.
7. The method of claim 5 wherein said variant hybridizes to at least one probe of Table I under conditions of moderate stringency.
8. The method of claim 2 wherein expression of at least one RNA transcript is detected or determined by at least one member of the group consisting of PCR, Northern Analysis, and in situ hybridization.

20110110 16562660

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

9. The method of claim 2 wherein expression of said at least two RNA transcripts are detected by a DNA array.
10. A database comprising a multiplicity of nucleotide sequences shown in any one of Table I, including variants thereof, wherein said variants hybridize under conditions of high stringency to either strand of a denatured, double-stranded DNA comprising any of SEQ ID NOS: 1-327.
11. The database of claim 10 wherein said variants hybridize under conditions of moderate stringency.
12. A DNA array comprising a multiplicity of nucleotide sequences shown in Table I, including variants thereof, wherein said variants hybridize under conditions of high stringency to either strand of a denatured, double-stranded DNA comprising any of SEQ ID NOS: 1-327.
13. The DNA array of claim 12 wherein said variants hybridize under conditions of moderate stringency.
14. A method for staging plant embryos comprising:
 - a) selecting total RNA from a multiplicity of embryos of known developmental age;
 - b) correlating the embryonic expression pattern to the developmental age to develop a relational database;
 - c) determining levels of expression from embryos of unknown developmental age by hybridization to a DNA array comprising a multiplicity of the nucleotide sequences shown in any one of SEQ ID NOS: 1-327;
 - d) correlating the expression pattern from step 3 to the relational database to determine developmental stage for the unknown embryo.

20470"4662650

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

15. The method of claim 14 wherein the embryos of step 1) are zygotic embryos.
16. The method of claim 14 further comprising the step of altering the embryonic growth conditions to approximate the expression pattern of zygotic embryos.
17. An isolated nucleic acid variant of the nucleotide sequence shown in any one of SEQ ID NOS: 1-334, wherein said variant hybridizes under conditions of moderate stringency to either strand of a denatured, double-stranded DNA comprising any of SEQ ID NOS: 1-334.
18. An isolated polypeptide encoded by a nucleic acid molecule of claim 17.
19. An isolated nucleic acid encoding the polypeptide of claim 18.
20. Antibodies that specifically bind to the peptide of claim 18.
21. The antibodies of claim 20, wherein said antibodies are monoclonal.
22. A recombinant vector that directs the expression of a nucleic acid of claim 17.
23. A host cell transformed with the vector of claim 22.
24. The host cell of claim 23, wherein the host is a somatic pine embryo.
25. A method for staging plant embryos comprising:
 - a) selecting total RNA from at least one embryo of known developmental age;
 - b) determining the level of expression of a multiplicity of genes which hybridize to one or more of SEQ ID NOS: 1-327;

2004-04-04 16:04:00

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

- c) correlating the known developmental ages of the embryos from step 1) with the profile of expression measured in step 2);
 - d) applying the correlation of step 3) to a sample of embryo RNA from embryos to be staged; and
 - e) determining the embryo stage.
26. The method of claim 25, wherein the measurement of gene expression is by RT-PCR.
27. The method of claim 25, wherein the measurement of gene expression is by nucleic acid hybridization.
28. The method of claim 25, wherein the measurement of gene expression is by determining the level of protein expression.
29. The method of claim 28, wherein protein expression is measured by antibody binding.
30. A method for selecting advantageous plant clones comprising:
- a) selecting one or more samples of embryonic RNA from multiple clones of plants;
 - b) determining that at least one sampled clone has an advantageous characteristic;
 - c) comparing the embryonic levels of expression of genes which hybridize to one or more of SEQ ID NOS: 1-327 in samples from the advantageous clone with expression levels in at least one clone that does not show the advantageous characteristic; and
 - d) selecting additional clones which show an embryonic gene expression pattern more similar to that of the advantageous clone than to the pattern of at least one clone that does not show the advantageous characteristic.

2014-04-04 10:00:00

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

31. Method of claim 30 where the clones to be sampled or compared are from about the same developmental age.
32. Method of claim 31 where the development age is visually detected.
33. The method of claim 30, wherein the measurement of gene expression is by RT-PCR.
34. The method of claim 30, wherein the measurement of gene expression is by nucleic acid hybridization.
35. The method of claim 30, wherein the measurement of gene expression is by determining the level of protein expression.
36. The method of claim 35, wherein protein expression is measured by antibody binding.
37. A method of determining embryo fitness comprising:
 - a) creating a relational database with RNA expression values for genes listed in Table I for embryos of known developmental stages;
 - b) isolating total RNA from embryos of unknown stage development;
 - c) measuring expression levels of genes identified in Table I from the isolated total RNA; and
 - d) correlating the database of step 1) with the pattern of expression determined in steps 2) and 3) to assess proper embryo development.
38. The method of claim 37, wherein the measurement of gene expression is by RT-PCR.

2024-04-06 16:25:50

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

39. The method of claim 37, wherein the measurement of gene expression is by nucleic acid hybridization.
40. The method of claim 37, wherein the measurement of gene expression is by determining the level of protein expression.
41. The method of claim 40, wherein protein expression is measured by antibody binding.
42. A method for selecting advantageous growth conditions for embryo development comprising:
 - a) determining RNA expression profiles for staged embryos under control culture conditions;
 - b) altering culture conditions;
 - c) determining RNA expression profiles for staged embryos under altered culture conditions; and
 - d) correlating culture change to developmental effect in embryo.
43. The method of claim 42, wherein conditions are selected which produce RNA expression profiles most closely approximating late-stage embryo profiles.
44. The method of claim 42, wherein the culture conditions are altered by operatively linking one or more stage-specific embryo promoter(s) to one or more sense or antisense nucleic acid molecules.
45. The method of claim 42, wherein the culture conditions are altered by operatively linking one more stage-specific embryo promoter(s) selected from SEQ ID NOS: 328-334 to one or more sense or antisense nucleic acid molecules.

2014-04-01 10:00:00

FINNEGAN
 HENDERSON
 FARABOW
 GARRETT &
 DUNNER LLP

1300 I Street, NW
 Washington, DC 20005
 202.408.4000
 Fax 202.408.4400
 www.finnegan.com

46. The method of claim 42, wherein the change in expression profiles is correlated by a relational database.
47. A recombinant nucleic acid molecule encoding a product during embryo development comprising:
- a) a first nucleic acid sequence which is the LP2-3 promoter; and
 - b) a second nucleic acid sequence encoding a product, wherein the first nucleic acid is operatively linked to the second nucleic acid molecule whereby its expression is directed by the promoter sequence.
48. The recombinant nucleic acid molecule of claim 47 wherein the second nucleic acid sequence encodes for GFP, or a variant of GFP.
49. The recombinant nucleic acid molecule of claim 48 wherein the second nucleic acid sequence is linked to one or more additional nucleic acid molecules.
50. The recombinant nucleic acid molecule of claim 49 wherein the additional molecule encodes a protein product normally expressed by a developing embryo at a known stage.
51. The recombinant nucleic acid molecule of claim 47 wherein the second nucleic acid sequence encodes an embryo-derived molecule.
52. The recombinant nucleic acid molecule of claim 51 embryo-derived molecule is stage-specific.
53. A plant cell comprising the recombinant nucleic acid molecule of claim 47.
54. A method for producing a protein product during embryo development comprising:
- a) operatively linking one more stage-specific embryo promoter(s) to one or

204 F.T.O. 4652, 4650

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com

- more nucleic acid molecules that encode a protein product,
- b) delivering construct to developing embryos.

55. The method of claim 54 wherein the operatively linked nucleic acid molecule is a reporter or indicator gene.
56. The method of claim 54 wherein the operatively linked nucleic acid molecule is GFP, or a variant of GFP
57. The method of claim 54 wherein at least one stage-specific promoter is selected from SEQ ID NOS: 328-334.
58. A method for staging embryos comprising:
 - a) providing one or more stage-specific embryo promoter(s) operatively linked to one or more nucleic acid molecules that encode a protein product to developing embryos,
 - b) monitoring expression of the protein product as the embryo matures through stage in which promoter functions.
59. The method of claim 58 wherein the operatively linked nucleic acid molecule is a reporter or indicator gene.
60. The method of claim 58 wherein the operatively linked nucleic acid molecule is GFP, or a variant of GFP.
61. The method of claim 58 wherein at least one stage-specific promoter is selected from SEQ ID NOS: 328-334.

2017-04-06 16:00

FINNEGAN
HENDERSON
FARABOW
GARRETT &
DUNNER LLP

1300 I Street, NW
Washington, DC 20005
202.408.4000
Fax 202.408.4400
www.finnegan.com